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National Poultry Improvement Plan

Proposed Changes To Be Considered At The National Plan Conference

Colorado Springs, Colorado

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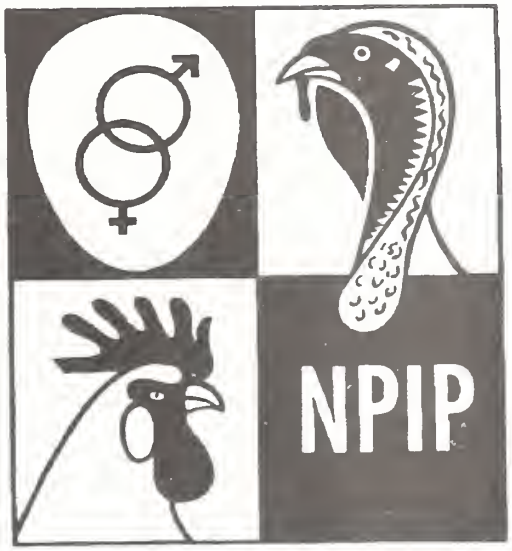
57 Years of Poultry Improvement



NATIONAL POULTRY IMPROVEMENT PLAN

IN THE
NATIONAL POULTRY IMPROVEMENT PLAN

INTRODUCTORY STATEMENT

Present provisions of the National Poultry Improvement Plan are contained in the U.S. Department of Agriculture publication, "National Poultry Improvement Plan and Auxiliary Provisions," APHIS 91-40, 1989 and in Title 9 CFR parts 145 and 147.

The detailed procedure for making changes in the Plan is described in the auxiliary provisions, sections 147.41 through 147.48. Copies of the "National Poultry Improvement Plan and Auxiliary Provisions" are available from each Official State Agency or from the National Poultry Improvement Plan staff, Animal and Plant Health Inspection Service, Veterinary Services, Room 771, 6505 Belcrest Road, Hyattsville, Maryland 20782.

Proposed changes and supporting statements in this publication were submitted as provided in section 147.44. They are compiled in this publication for consideration at the 1992 National Plan Conference. This publication is distributed well in advance of the conference so that participants and other interested persons may review the proposed changes and inform conference delegates of their wishes regarding the proposals.

Some proposed changes have a line drawn through a portion of the words while other portions are underscored. The line through the words indicate that they are part of the present provision but would be deleted if the proposal were adopted. The underscored words are the proposed additions to that provision.

Each State is entitled to one official delegate for each of the subparts, B, C, D, and E of part 145 in which it has one or more participants at the time of the conference. Each delegate will act on proposals affecting the provisions of the program which he represents. For reference purposes, delegates are designated as follows:

subpart B delegates - representing egg-type chickens.
subpart C delegates - representing meat-type chickens.
subpart D delegates - representing turkeys.
subpart E delegates - representing waterfowl, exhibition poultry, and game birds.

This compilation of proposed changes includes, in the margin adjacent to the section reference for each proposal, the delegate entitled to vote on the proposal. Some of the changes proposed apply equally to all participants in which case conference action will be determined by the combined vote of all delegates.

Section numbers shown with each proposal refer to the numerical identification of affected provisions as published in "National Poultry Improvement Plan and Auxiliary Provisions," APHIS 91-40, August 1989. A review of the listing of section numbers and titles may help in locating the provisions involved.

PROPOSED CHANGES
IN THE
NATIONAL POULTRY IMPROVEMENT PLAN

Contents

9	(B,C,E)	18	Removes the provision that allows two generations to go without blood testing for Pullorum-Typhoid
10	B	19	Provides for various sample sizes of live birds for bacteriological examination under the U.S. Sanitation Monitored Program for Egg-Type Chickens.
11	(B,C,D,E)	22	Amends the procedure recommended for the bacteriological examination of Salmonella reactors.
12	(B,C,D,E)	28	Amends the procedures for collecting environmental samples and cloacal swabs for bacteriological examination.
13	(B,C,D,E)	35	Amends the procedure that determines status and effectiveness of sanitation monitored program.
14	(B,C,D,E)	37	Amends Pullorum-Typhoid testing requirements for reactor flocks.
15	C	39	Provides for 100% Pullorum-Typhoid testing of male-line grandparent flocks.
16	(B,C,D,E)	40	Provides for the modification of Mycoplasma Hemagglutination Inhibition laboratory protocol.
17	B	45	Proposes a voluntary program for S. enteritidis tested started poultry.
18	(B,C,D,E)	48	Proposes changes in the procedure in the isolation and identification procedures of <u>Salmonella pullorum.</u>

19	B	53	Proposes to change §145.23 (d) to U.S. S. enteritidis Monitored.

Proposal No. 1

Delegates:
B

§ 145.23 (d) U.S. SANITATION MONITORED. Amends the provisions as follows

(d) **U.S. Sanitation Monitored.** This program is intended to be the basis from which the breeding-hatching industry may conduct a program for the prevention and control of Salmonellosis. It is intended to reduce the incidence of Salmonella organisms in hatching eggs and chicks through an effective and practical sanitation program at the breeder farm and in the hatchery. This will afford other segments of the poultry industry an opportunity to reduce the incidence of Salmonella in their products.

(d)(1) A flock and the hatching eggs and chicks produced from it which have met the following requirements as determined by the Official State Agency:
(d) (1) (i) The flock originated from a U.S. Sanitation Monitored flock, or meconium from the chick boxes and a sample of chicks that died within 7 days after hatching are examined bacteriologically for salmonella at an authorized laboratory. Cultures from positive samples shall be serotyped.
(d)(1)(ii) All feed fed to the flock shall meet the following requirements:

(d)(1)(ii)(A) If pelletized feed contains animal protein, the protein products should be purchased from participants in the Animal Protein Products Industry (APPI) Salmonella Education/ Reduction Program, with a minimum moisture content of 14.5 percent, and must have been subjected to temperature of 190° F. or above, 165°F. for at least 20 minutes, or 184°F. and 70 lbs. of pressure during the manufacturing process.
(d)(1)(ii)(B) If mash feed contains animal protein, the protein products should be purchased from participants in the Animal Protein Producers Industry (APPI) Salmonella Education/Reduction Program.
(d)(1)(iii) Feed shall be stored and transported in such a manner as to prevent possible contamination;

(d)(1)(iv) The flock is maintained in compliance with §§ 147.21, 147.24 (a), and 147.26 of this chapter;
(d)(1)(v) Environmental samples shall be collected from the flock by an Authorized Agent, as described in § 147.12 of this chapter, when the flock is more than ~~4 months~~ 8 weeks of age **and every 30 days thereafter**. The samples shall be examined bacteriologically for group D salmonella at an authorized laboratory. Cultures from positive samples shall be serotyped.
(d)(1)(vi) A federally licensed Salmonella enteritidis bacterin may be used in multiplier breeding flocks except for a sample of 350 birds, which will be banded for identification, which are negative for Salmonella enteritidis on bacteriological examination as described in (d)(1)(v) of this section. Following negative serological and bacteriological examinations as described in (d)(1)(vii) the banded non-vaccinated birds shall be vaccinated.
(d)(1)(vii) Blood samples from 300 non-vaccinated birds as described in (d)(1)(vi) shall be officially tested with pullorum antigen when the flock is a minimum of more than 4 months of age. All birds with positive or inconclusive reactions, up to a maximum of 25 birds, shall be submitted to an authorized laboratory and examined for the presence of group D salmonella, as described in § 147.11 of this chapter. Cultures from positive samples shall be serotyped.
(d)(1)(viii) Hatching eggs are collected as quickly as possible and are handled as described in § 147.22 of this chapter and sanitized or fumigated as described in § 147.25(a) of this chapter.
(d)(1)(ix) Hatching eggs produced by the flock are incubated in a hatchery that is in compliance with the recommendations in §§ 147.23 and 147.24(b) of this chapter, and sanitized either by a procedure approved by the Official State Agency or as prescribed in § 147.25 of this chapter.
(d)(2) A flock shall not be eligible for this classification if Salmonella enteritidis (S. enteritidis ser Enteritidis) is isolated from a specimen taken from a bird in the flock. Isolation of SE from an environmental or other specimen as described in section (d)(1)(v) of

this paragraph will require that a random sample of 60 live birds will be examined bacteriologically for Salmonella in an authorized laboratory as described in section 147.11 of this chapter. If a sample is found positive for SE, the flock certification will be temporarily suspended and at the discretion of the Official State Agency another 60 bird sample shall be submitted within 10 days for culture.

~~(d)(3) A flock shall be eligible for this classification if **Salmonella enteritidis** (S. enteritidis ser Enteritidis) is isolated from an environmental sample collected from the flock in accordance with paragraph (d)(v) of this section: Provided, That testing is conducted in accordance with paragraph (d)(1)(vii) of this section each 30 days and no positive samples are found.~~

(d)(4) In order for a hatchery to sell products of this classification, all products handled shall meet the requirements of the classification.

(d)(5) This classification may be revoked by the Official State Agency if the participant fails to follow recommended corrective measures.

REASON:

The General Conference Committee thought that this change would provide preventive protection for negative multiplier egg-type chicken breeding flocks from chance extraneous sources of SE infection such as feed and environmental sources i.e. other feral animals, birds and humans. This would also give added insurance that all chicks would be free of SE without seriously compromising any detection system.

PROPONENT:

General Conference Committee, National Poultry Improvement Plan.

Proposal No. 2

Delegates:

B,C,D,E (combined)

§145.1 DEFINITIONS. Adds the following definition.

Suspect flock. A flock from which Salmonella pullorum, Salmonella gallinarum, Salmonella enteritidis, M. gallisepticum, M. synoviae, and/or M. meleagridis has been isolated from the flocks offspring or hatchmates or by other epidemiological evidence that flock may have been exposed.

REASON:
This change was considered important enough to incorporate into the provisions of the Plan, based on experiences with a pullorum outbreak in a large multi-state meat-type chicken integrator. This additional definition would aid in identifying all the flocks that should be investigated in order for the complete eradication of pullorum during an outbreak.

PROPONENT:
Dr. Kenneth G. Keenum, DVM, Assistant Director of Laboratories, Rollins Animal Disease Diagnostic Laboratory, North Carolina Department of Agriculture, Raleigh, NC.

Proposal No. 3

Delegates:

B,C,D,E (combined)

§ 145.14 (a) BLOOD TESTING FOR PULLORUM-TYPHOID. This amends as follows.

(a)(1) FOR PULLORUM-TYPHOID. The official blood tests for pullorum-typhoid shall be the standard tube agglutination test, the microagglutination test, the enzyme-labeled immunosorbent assay test (ELISA), or the rapid serum test for all poultry; and the stained antigen, rapid whole-blood test for all poultry except turkeys. The procedures for conducting official blood tests are set forth in §§ 147.1, 147.2, 147.3, and 147.5 of this chapter and referenced in footnote 3 of this section. Only antigens approved by the Department and of the polyvalent type shall be used for the rapid whole-blood and tube agglutination test. The tube antigen should be approved by the Department by submission of each lot made by the State or once a year to the Department. All microtest antigens and enzymelabeled immunosorbent assay reagents shall also be approved by the Department.

(a)(6) The taking of blood samples--performed by or in the presence of a State inspector--from all birds (100%) on premises exposed to birds, equipment, supplies, or personnel (suspect flock) from the primary breeding flock during the period when the State Inspector determines that exposure to S. pullorum or S. gallinarum occurred.

REASON:
The tube agglutination test is used to retest serum from positive reaction to the Rapid Whole-Blood test in the field. If the reaction to this retest is positive in dilutions of 1:50 or greater for the standard tube agglutination test, additional examination of the bird and flock will be performed. A standard or approval of the TA antigen is critical to an effective program.

PROPONENT:

Dr. Kenneth G. Keenum, DVM, Assistant Director of Laboratories, Rollins Animal Disease Diagnostic Laboratory, North Carolina Department of Agriculture, Raleigh, NC

Proposal No. 4

Delegates:

B,C,D,E (Combined)

§ 145.23 (b)(3)(v) Change section to read:
§ 145.33 (b)(3)(v) Change section to read:
§ 145.43 (b)(3)(v) Change section to read:
§ 145.53 (b)(3)(v) Change section to read:

All reports of S. pullorum or S. gallinarum isolations from poultry are promptly followed by an investigation by the Official State Agency to determine the origin of the infection; provided that if the origin of the infection involves another State or if there is possible exposure to another State from the positive flock, then the Service will promptly investigate.

REASON:
Interstate movement of suspect progeny should be investigated by the Service in order to facilitate the determination of the source of the infection and swift eradication with as little industry disruption as possible.

PROPONENT:
Dr. Kenneth G. Keenum, DVM, Assistant Director of Laboratories, Rollins Animal Disease Diagnostic Laboratory, North Carolina Department of Agriculture, Raleigh, NC

Proposal No. 5

Delegates:
B,C,D,E
(combined)

§ 145.23(b)(5) Clarification of testing
§ 145.33(b)(5) Clarification of testing
§ 145.43(b)(5) Clarification of testing
§ 145.53(b)(5) Clarification of testing

(b)(5) It is a primary breeding flock located in a State determined to be in compliance with the provisions of paragraph (b)(4) of this section, and that great-grandparent/pedigree flocks test each bird in the flock for pullorum-typhoid with no reactors when the flock is at 25% production. Grandparent flocks - test 10% of flock at 25% production and retest 10% of flock at 40-45 weeks of age. ~~in which a sample of 300 birds from flocks of more than 300, and each bird in flocks of 300 or less, has been officially tested for pullorum-typhoid with no reactors:~~ Provided, That a bacteriological examination monitoring program acceptable to the Official State Agency and approved by the Service may be used in lieu of blood testing.

REASON:
This requires a more thorough testing of great-grandparent and pedigree stock and a retest for grandparent stock since they are likely to be on contract farms at 40-45 weeks of age.

PROPONENT:
Dr. Kenneth G. Keenum, DVM, Assistant Director of Laboratories, Rollins Animal Disease Diagnostic Laboratory, North Carolina Department of Agriculture, Raleigh, NC

Proposal No. 6

Delegates:
B,C,D,E

(combined)

§§ 145.23(b)(5), 145.33(b)(5), 145.43(b)(5), 145.53 (b)(5). Deletes sample testing of Primary breeders.

(b)(5) It is a primary breeding flock located in a State determined to be in compliance with the provisions of paragraph (b)(4) of this section, and in which 100% of the birds have been officially tested for pullorum-typhoid with no reactors: ~~in which a sample of 300 birds from flocks of more than 300, and each bird in flocks of 300 or less, has been officially tested for pullorum-typhoid with no reactors:~~ Provided, That a bacteriological examination monitoring program acceptable to the Official State Agency and approved by the Service may be used in lieu of blood testing.

REASON:
When a flock is a suspect flock: 1. It was hatched in the same hatchery as a Pullorum positive flock; 2. It is related to flocks that are Pullorum positive or have produced Pullorum positive progeny; 3. It produces Pullorum positive progeny; or 4. It tests positive on the WB or TA test, then a 100% test is required for all suspect flocks. This may require 100% testing of 100,000 or more multiplier breeders. The testing of 100% of all primary breeders may eliminate the need for such extensive testing in the event of a pullorum outbreak.

PROPONENT:
Dr. Martha L. Ewing, DVM, Veterinary Manager, Bureau of Contagious and Infectious Diseases, Florida Department of Agriculture & Consumer Services, The Capitol, Tallahassee, FL 32399

Proposal No. 7

Delegates:
B.C,D,E
(combined)

§ 145.14(a)(10) amends as follows:

(a)(10) Any drug, for which there is scientific evidence of masking the reaction or hindering the bacteriological recovery of salmonella organisms, shall not be fed or administered to poultry within 3 weeks prior to a test or bacteriological examination for pullorum-typhoid, provided that when suspect multiplier-breeder flocks, (flocks that are the progeny of a pullorum-typhoid positive flock, flocks that have been exposed to a positive flock, or flocks whose progeny has been determined to be pullorum-typhoid positive), have been identified, the Official State Agency may, at its discretion, allow the participant to medicate such poultry in lieu of the 100% testing requirements. Chicks from such flocks must be maintained under strict quarantine and only be used for slaughter purposes. The participant is responsible for adhering to FDA regulations concerning drug withdrawal.

REASON:
In the event of an outbreak, it may be advantageous for a producer to medicate multiplier and grow-out flocks in an effort to cut losses. However, medicating primary breeding flocks can be very dangerous. Such treatments may mask problems that will become apparent in the highly stressed environment of end-line production flocks, and interfere with diagnosing the disease.

Proponent:
Dr. Martha L. Ewing, DVM, Veterinary Manager, Bureau of Contagious and Infectious Diseases, Florida Department of Agriculture & Consumer Services, The Capitol, Tallahassee, FL 32399

Proposal No. 8

Delegates:
E

§ 145.52 Participation Attempts to reduce paperwork load.

§ 145.52 Participation.
Participating flocks of waterfowl, exhibition poultry, and game birds, and the eggs and baby poultry produced from them shall comply with the applicable general provisions of Subpart A of this part and the special provisions of this Subpart E.
(a) Poultry products produced under Subpart "E" shall comply with 145.4 and may use one of the following to report poultry sales to importing States.

(1) NPIP VS 9-3 Form, or

(2) Approved number on shipping label and billing invoice, or

(3) Timely computer printouts of sales to each State, subject to Official State Agency and Departmental approval.

~~(a)~~(b) Started poultry shall lose their identity under Plan terminology when not maintained by Plan participants under the conditions prescribed in § 145.5 (a).
~~(b)~~(c) Hatching eggs produced by primary breeding flocks shall be fumigated as described in § 147.25 or otherwise sanitized.

REASON:
Reduce time and paper work necessary for each poultry sale.

PROPONENT:
Mr. Monroe Fuchs, Ideal Poultry Breeding Farms, Inc., P.O. Box 591, Cameron, TX 76520 and Texas Poultry Improvement Board.

Proposal No. 9

Delegates:
B,C,E

§ 145.23(b)(3) Amends as follows
§ 145.33(b)(3) Amends as follows
§ 145.53(b)(3) Amends as follows
(b)(3) It is a multiplier breeding flock, ~~or a breeding flock composed of progeny of a primary breeding flock which is intended solely for the production of multiplier breeding flocks~~, that originated from U.S. Pullorum-Typhoid Clean breeding flocks or from flocks that met equivalent requirements under official supervision, and is located in a State in which it has been determined by the Service that:

REASON:
Pullorum disease and/or fowl typhoid can be introduced and infection established at any level of primary breeder flocks following lapses in basic biosecurity. When two (2) generations of infected breeder flocks are allowed to go untested, wide distribution of pullorum disease and/or fowl typhoid can occur before detection.

PROPONENT:
Dr. George Stein, Jr., Laboratory Director, Animal Health Laboratory, Maryland, Department of Agriculture, P.O. Box J, Salisbury, MD 21802

Proposal No. 10

Delegates:
B

§ 145.23 (d) Amends the sample size for live birds as follows:

(d) **U.S. Sanitation Monitored.** This program is intended to be the basis from which the breeding-hatching industry may conduct a program for the prevention and control of Salmonellosis. It is intended to reduce the incidence of Salmonella organisms in hatching eggs and chicks through an effective and practical sanitation program at the breeder farm and in the hatchery. This will afford other segments of the poultry industry an opportunity to reduce the incidence of Salmonella in their products.

(d)(1) A flock and the hatching eggs and chicks produced from it which have met the following requirements as determined by the Official State Agency:

(d) (1) (i) The flock originated from a U.S. Sanitation Monitored flock, or meconium from the chick boxes and a sample of chicks that died within 7 days after hatching are examined bacteriologically for salmonella at an authorized laboratory. Cultures from positive samples shall be serotyped.

(d)(1)(ii) All feed fed to the flock shall meet the following requirements:

(d)(1)(ii)(A) If pelletized feed contains animal protein, the protein products should be purchased from participants in the Animal Protein Products Industry (APPI) Salmonella Education/ Reduction Program, with a minimum moisture content of 14.5 percent, and must have been subjected to temperature of 190° F. or above, 165°F. for at least 20 minutes, or 184°F. and 70 lbs. of pressure during the manufacturing process.

(d)(1)(ii)(B) If mash feed contains animal protein, the protein products should be purchased from participants in the Animal Protein Producers Industry (APPI) Salmonella Education/Reduction Program.

(d)(1)(iii) Feed shall be stored and transported in such a manner as to prevent possible contamination;

(d)(1)(iv) The flock is maintained in compliance with §§ 147.21, 147.24 (a), and 147.26 of this chapter;
(d)(1)(v) Environmental samples shall be collected from the flock by an Authorized Agent, as described in § 147.12 of this chapter, when the flock is more than 4 months of age **and every 30 days thereafter**. The samples shall be examined bacteriologically for group D salmonella at an authorized laboratory. Cultures from positive samples shall be serotyped.
(d)(1)(vi) Blood samples from 300 birds shall be officially tested with pullorum antigen when the flock is a minimum of more than 4 months of age. All birds with positive or inconclusive reactions, up to a maximum of 25 birds, shall be submitted to an authorized laboratory and examined for the presence of group D salmonella, as described in § 147.11 of this chapter. Cultures from positive samples shall be serotyped.
(d)(1)(vii) Hatching eggs are collected as quickly as possible and are handled as described in § 147.22 of this chapter and sanitized or fumigated as described in § 147.25(a) of this chapter.
(d)(1)(viii) Hatching eggs produced by the flock are incubated in a hatchery that is in compliance with the recommendations in §§ 147.23 and 147.24(b) of this chapter, and sanitized either by a procedure approved by the Official State Agency or as prescribed in § 147.25 of this chapter.
(d)(2) A flock shall not be eligible for this classification if Salmonella enteritidis (S. enteritidis ser Enteritidis) is isolated from a specimen taken from a bird in the flock. Isolation of SE from an environmental or other specimen as described in section (d)(1)(v) of this paragraph will require that a random sample of 60 live birds from a flock over 5000 birds, 30 live birds from a flock between 2,000 and 4,999 birds, and 20 birds from a flock under 2,000 birds will be examined bacteriologically for Salmonella in an authorized laboratory as described in section 147.11 of this chapter. If a sample is found positive for SE, the flock certification temporarily suspended and at the discretion of the Official State Agency another 60 bird

sample shall be submitted within 10 days for culture.
(d)(3) A flock shall be eligible for this classification if **Salmonella enteritidis** (S. enteritidis ser Enteritidis) is isolated from an environmental sample collected from the flock in accordance with paragraph (d)(v) of this section: Provided, That testing is conducted in accordance with paragraph (d)(1)(vi) of this section each 30 days and no positive samples are found.
(d)(4) In order for a hatchery to sell products of this classification, all products handled shall meet the requirements of the classification.
(d)(5) This classification may be revoked by the Official State Agency if the participant fails to follow recommended corrective measures.

REASON:
The current requirement for U.S. Sanitation Monitoring states that if a positive (SE) isolate from environmental sampling occurs, a sixty bird random sample of the flock for laboratory analysis must be taken. The sixty bird sampling size is standard, regardless of the flock size, whether it is 100 birds or 100,000 birds).
I feel that the small commercial flock owner or small egg type hatchery is at an disadvantage by having to sacrifice sixty birds. I suggest that statistically sampling would be more realistic. Simply follow a similar guideline you use for the number of samples required on National Poultry Improvement Plan Provisions 147.12 (a)(2).

PROPONENT:
Mr. Les Pulfer, State of Illinois, Department of Agriculture, Division of Animal Industries, State Fairgrounds, P.O. box 19281, Springfield, IL 62794

Proposal No. 11

Delegates:

B,C,D,E (combined)

§147.11 Amends as follows and adds Illus. 1&2:

§147.11 **LABORATORY PROCEDURE RECOMMENDED FOR THE BACTERIOLOGICAL EXAMINATION OF SALMONELLA REACTORS.**

Pullorum-Typhoid and other Salmonella reactors or suspects shall be cultured in accord with both (a) direct and (b) selective enrichment procedures described in this section. Careful aseptic technique must be used when collecting all tissue samples.

(a) Direct Culture (refer to Illus. 1) Grossly normal or diseased liver, heart, pericardial sac, spleen, lung, kidney, pancreas, peritoneum, drained gall bladder, ~~or~~, oviduct~~,~~ misshapen ova or testes~~,~~ inflamed or unabsorbed yolk sac~~,~~ and other visibly pathological tissues where purulent, necrotic, or proliferative lesions are seen (including cysts, abscesses, hypopyon, and inflamed serosal surfaces)~~,~~ ~~should~~ shall be ~~directly cultured by means of a~~ sampled for direct culture using either flamed wire loops or ~~with~~ sterile swabs[1]. ~~Careful aseptic techniques must be utilized throughout the process of collecting tissues. Selective media should not be relied upon to deal with contaminants,~~ Since some strains may not dependably survive and grow in certain selective media, inoculate non-selective broths and plates in addition to two (2) different selective plating media. Refer to Illus. 1 for required bacteriological recovery and identification procedures[1]. Proceed immediately with collection of organs and tissues for selective enrichment culture. ~~Inoculate veal infusion (VI) and brilliant~~

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[1]~~Culture media preparations and~~ Biochemical identification charts can be obtained from ~~Culture Methods for the Detection of Animal Salmonellosis and Arizonosis, Committee on Salmonellosis and Arizonosis; AAVLD; 1976 Iowa State University Press; Ames, Iowa 50010.~~ A Laboratory Manual for the Isolation and Identification of Avian Pathogens. Chapter 1. Salmonellosis. Third Edition, 1989, American Association of Avian Pathologists, Inc., Kendall/Hunt Publishing Co., Dubuque, Iowa 52004-0539.

~~green (BG) agar plates. Inoculate the plates for 24 and 48 hours at 37°C. The digestive system should always be cultured separately (see paragraph (f) of this section) after other anatomical organs and systems have been collected and cultured.~~

(b) Selective Enrichment Culture (refer to Illus. 2). Collect and culture organ samples separately from intestinal samples, with intestinal tissues collected last to prevent cross-contamination. Samples from the following organs or sites should be ~~aseptically~~ collected for culture in selective enrichment broth:

(b)(1) no change;
(b)(2) " " ;
(b)(3) " " ;
(b)(4) " " ;
(b)(5) " " ; and
(b)(6) spleen and other visible pathological sites where purulent, necrotic, or proliferative lesions are seen.

(c) From each reactor or suspect, aseptically collect 10-15 g or ~~whatever~~ the nearest lesser amount ~~is~~ available ~~of~~ from each organ or site listed in paragraph (b) ~~from each reactor~~, and mince, grind ~~or~~ and blend them completely in ~~10 time their volume of VI broth~~ sufficient small quantities of beef extract broth, or a comparable non-selective broth, to produce an easily poured suspension. Organs ~~may be processed individually or in combinations where appropriate~~ or sites listed in paragraph (b) of this section may be pooled with those from other reactors or suspects, but not to exceed five (5) reactors or suspects per pool. ~~Suspensions should be transferred in 10 ml aliquots to 100 ml of both VI and tetrathionate brilliant green (TBG) broth and incubated at 37°C for 24 hours. Plate the VI broth on VI and BG agar and plate the TBG broth on BG agar and incubate at 37°C. Examine these plates after 24 and 48 hours of incubation. The contents of the gallbladder can be cultured separately by inoculating 10 ml of VI and TBG broth with cotton swabs and incubating at 37°C for 24 hours. Plate on BG agar and incubate at 37°C. Examine these plates after 24 and 48 hours of incubation. If contamination with pseudomonas and proteus~~

~~is a problem, make platings on BG sulfapyridine (BGS) agar~~. Ten ml aliquots of each suspension pool shall be inoculated (transferred) into a 100 ml volume of selective enrichment broth (1:10 ratio, sample suspension: broth). Refer to Illus.2 for required bacteriological recovery and identification procedures, including delayed secondary enrichment.

~~(d) Where field samples are directly inoculated into enrichment broths and a delay of several days occurs before they reach a laboratory, or if recovery of low numbers or organisms is expected from a primary culture, a secondary enrichment culture should be prepared. Subculture a week-old primary culture by transferring 1 ml of inoculum into a fresh tube containing 10 ml of enrichment broth. This secondary enrichment should be incubated at 37°C for 24 hours before plating. (See paragraph (a) of this section.) TBG broth is recommended for this procedure.~~

~~(c)~~ (d) From each reactor or suspect, make a composite sample of the following parts of grossly normal or diseased tissues from the digestive tract: crop wall, duodenum, jejunum (including remnant of yolk-sac attachment), both ceca, cecal tonsils, and rectum-cloaca. Aseptically collect 10-15 g or ~~whatever~~ the nearest lesser amount ~~is~~ available ~~of~~ from each ~~organ or~~ specified digestive or intestinal tissue and mince, grind ~~or~~ and blend them completely ~~in 10 times their volume of TGB broth~~ to produce and easily poured suspension, as described in paragraph (c) of this section. The digestive/intestinal tissues may be pooled with similar tissues from other reactors or suspects, but not to exceed five (5) reactors or suspects per pool. ~~Transfer 10 ml of a composite sample of a suspension from the digestive tract into 100 ml of TBG broth, and incubate flasks at 42°C for 24 hours. Cultures may be incubated at 37°C if 42°C incubators are not available. The higher incubation temperatures for TBG broth reduce populations of competitive contaminants common in gut tissue. Plate on BG agar and incubate at 37°C. Examine the plates after 24 and 48 hours of incubation. If contamination with pseudomonas and proteus is a problem make plating on BGS agar~~. Ten ml aliquots of each suspension pool shall be

inoculated into a 100 ml volume of selective enrichment broth (1:10 ratio, sample suspension: broth). Refer to Illus.2 for required bacteriological recovery and identification procedures, including delayed secondary enrichment.

~~(f) If preferred, individual cotton swab samples may also be taken from the upper, middle, and lower intestinal tract (including both ceca and the rectum-cloaca). Deposit swabs in 10 ml of TGB broth and incubate and plate as described in paragraph (e) of this section.~~

~~(g) Transfer suspect colonies to triple-sugar-iron (TSI) agar and lysine-iron (LI) agar and incubate at 37°C for 24 hours.~~

~~(h) Cultures revealing typical reactions of salmonellae on TSI and LI agar slants should be transferred to any of the identification: Dextrose, lactose, sucrose, mannitol, maltose, dulcitol, malonate, gelatin, urea broth, citrate, lysine decarboxylase, ornithine, decarboxylase, methyl red and Voges-Proskauer, KCN, salicin broths, indole, and hydrogen sulfide. Motility or non-motility is demonstrated by inoculating a suitable semisolid medium.[2]~~

(e) The Analytical Profile Index for Enterobacteriaceae (API) system may be utilized to aid cultural ~~for~~ identifications ~~if feasible. For arizonae identification, make readings daily up to 10 days. An O-nitrophenyl-beta-D-galactopyranside (ONPG) disc may be used to identify slow lactose fermenters.[3]~~

~~(i)~~(f) All ~~salmonella cultures~~ isolates culturally identified as salmonellae should be ~~serologically typed~~ serogrouped or serotyped.

REASON:
The proposed changes seek to provide more clarity and detail for the collection and handling of organ and intestinal samples from

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[2]~~American Association of Avian Pathologists; University of Pennsylvania; New Bolton Center; Kennett Square, Pennsylvania 19348-1692, 1980.~~

[3]~~ONPG discs are available from: Baltimore Biological Laboratories; Cockeysville, Maryland 21030.~~

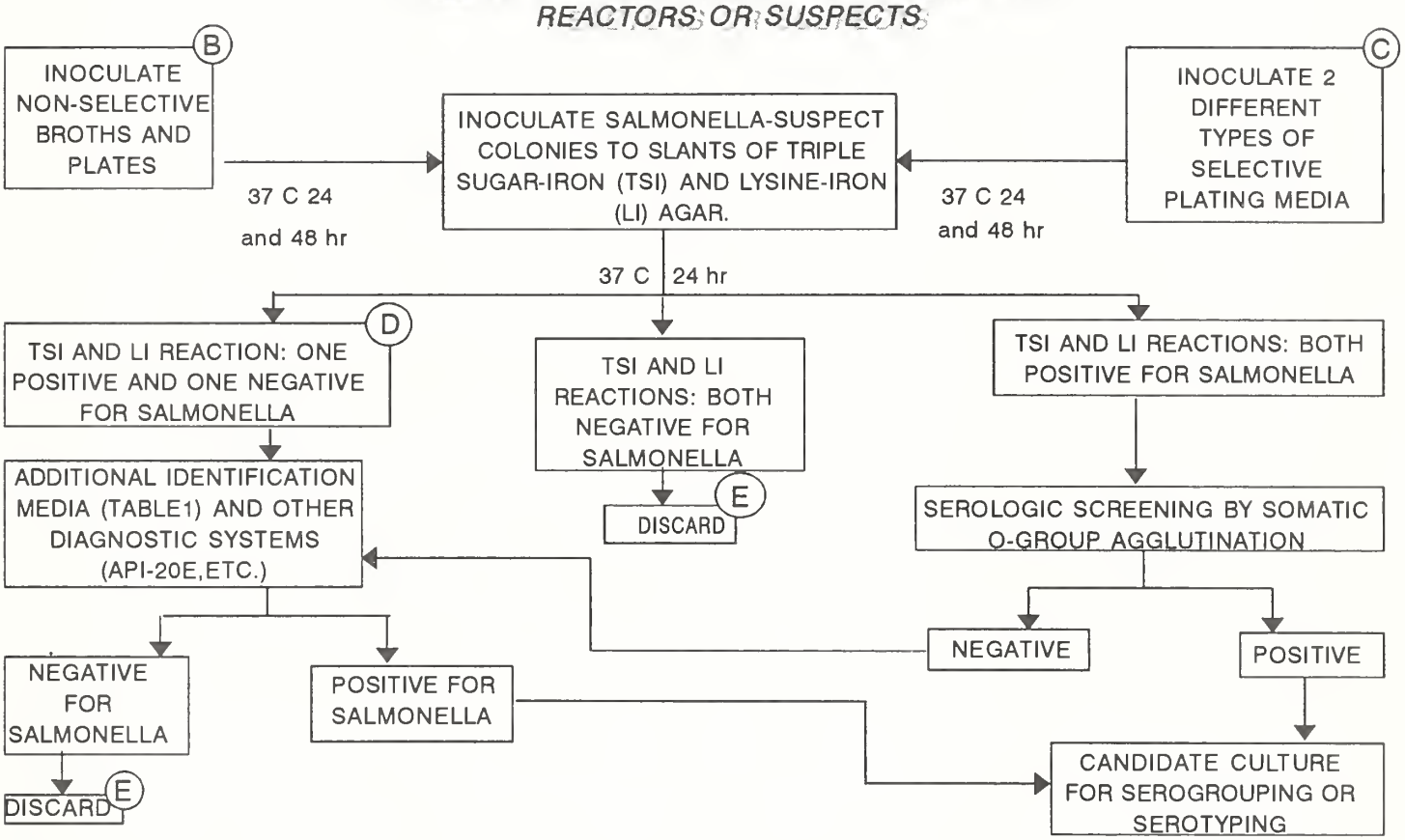
suspects or serological reactors cultured in NPIP Pullorum-Typhoid and Sanitation Monitored Programs. Specific bacteriological techniques for culturing these samples in both the older Pullorum-Typhoid Program and the more recent Sanitation Monitored Program are provided in a proposed flow chart format (Illus. 1 or 2) in this proposal. These illustrations update, and are intended to clarify, earlier NPIP and AAAP specifications.

PROPONENT: Dr. Ed Mallinson, VA-MD Regional College of Veterinary Medicine, University of Maryland, College Park, MD20742-3711.

Illus. 1

ORGAN (NON-INTESTINAL) TISSUES (A)

PULLORUM-TYPHOID AND OTHER SALMONELLA REACTORS OR SUSPECTS



A All pullorum-typhoid and other Salmonella reactors and suspects must also be evaluated with selective enrichment (refer to Illus 2)

B Inoculate both beef extract or infusion plates AND broths, or comparable non-selective plates and broths.

C For pullorum-typhoid reactors, inoculate brilliant green (BG) or BG novobiocin (BGN) AND another selective media such as xylose-lysine-desoxycholate. Other reactors or suspects, inoculate xylose-lysine-tergitol 4 AND BG or BGN.

D If combined results with TSI and LI agars, additional identification media, and O-group screening procedures are inconclusive, restreak original colony onto selective plating media to check for purity.

E Reevaluate if epidemiologic, necropsy, or other information strongly suggests the presence of an unusual strain of Salmonella.

Proposal No. 12

Delegates:
B,C,D,E
(combined)

§ 147.12 Procedures for Collecting Environmental Samples and Cloacal Swabs for Bacteriological Examination. Amends as follows:

Information concerning the pen arrangement and number of birds per pen should be obtained from the owner so that the required number of samples per pen and per flock can be determined. A means of identifying each sample by pen of origin should be provided. The vehicle transporting the personnel taking the samples should be left as far as practical from the poultry pens. Sanitary precautions, including personal cleanliness, should be observed during the sampling procedure. The hands should be carefully washed with a sanitizing soap prior to the sampling. Outer clothing, including gloves, should be changed between visits to different premises so that clean clothing is worn upon entering each premises.

The used and clean apparel should be kept separate. Boots or footwear should be cleaned and disinfected between visits to different premises. Disposable caps should be provided and discarded after use on each premises. After collection, the samples should be protected from drying, light, and excessive temperatures and delivered to the laboratory within one day. If delivery is delayed, samples should be refrigerated. All samples and swabs described in this section should be cultured in accord with Illus. 2, including delayed secondary enrichment. All salmonella recovered should be serogrouped or serotyped.

(a) Fecal material, litter, ~~or~~ dust, or litter surface or nest-box drag swab samples to be submitted for bacteriological examination should be collected in accordance with the procedures described in paragraphs (a)(1) or (2); and (c) and (c) (1) through (6) of this section:

(a)(1) Procedure for sampling in broth. Authorized laboratories will provide capped tubes 1-2 cm in diameter and 15-20 cm in length which are two-thirds full of recently

made, refrigerated, sterile selective enrichment broth (~~Selenite Brilliant Green Sulfapyridine~~ or Hajna or Mueller-Kauffman Tetrathionate Brilliant Green) for each sample. Sufficient tubes should be taken to the premises to provide at least one tube per pen or one tube per 500 birds, whichever is greater. At least one sterile, cotton-tipped applicator will be needed for each tube. The dry applicator is first placed or drawn through fresh manure (under roost, near water troughs, fecal droppings or diarrhetic droppings). After this and each subsequent streaking, the cotton-tipped applicator is placed in the tube of broth and swirled to remove the collected material. The applicator is then withdrawn and is used for taking additional specimens by streaking on or through areas where defecation, trampling of feces, or settling of dust is common; i.e., on or near waterers, feeders, nests, or rafters, etc. When the volume of material collected equals approximately 10 percent of the volume of the broth (usually 10-12 streakings), the applicator is placed in the tube and the stick is broken in half. The lower or cotton-tipped half is left in the broth, and the upper half is retained for future disposal. The cap is then replaced on the inoculated tube, and the sampling procedure is continued in other areas of the pen.

(a)(2) Procedure for sampling in dry containers. no changes
(b) Cloacal swabs. no changes.
(c) Drag-swabs. ~~Drag swabs should involve the exposure of at least four unpooled pads per house to promote representative sampling and some extent of quantification.~~ Utilization of drag-swabs (DS) involves the exposure of gauze pads, a key component of a DS sampler, to the surface of random, flock-representative litter and nest-box areas. The sampler pads should be sterile, and slightly moist to promote adherence of particulate material, and impregnated with non-inhibitory double-strength skim milk to protect salmonella viability during sample collection, batching, storage and shipment. Litter surface DS results tend to reflect the Salmonella carrier/shedder status of a flock.

Nonetheless, other environmental samples (nest-boxes, etc.), as described in paragraphs 147.12 (a)(1) or 147.12 (a)(2) and 147.12(c)(4) of this section, should also be periodically collected.

(c)(1) Drag-swab assembly. ~~Drag-swab~~ DS ~~sampling sets~~ sampler are assembled from two 3 x 3 cm sterile gauze pads that ~~were~~ are folded once and secured to various lengths of wrapping twine with paper clips, staples or similar fasteners. End wires of each paper clip are bent slightly to catch into the ~~swab~~ pad fabric, thus securing the clips to the folded pads. Two pads, assembled as described, are used to make each ~~drag-swab~~ DS ~~sampling set~~ sampler. With the paper clip attachment, one pad is securely connected through the free rounded end of the paper clip to a 2-ft (0.6 m) length of size 20 fibrous wrapping twine. The other pad is similarly connected to a 1 ft. (0.3 m) length of twine. Alternatively, the pads can also be securely attached to the twine by stapling the twine to the pad near the center of the fold in the pad. The staple is applied at a right angle to the twine and parallel to the fold. A pre-tied small tight knot in the free end of the twine, close and slightly distal to the staple-twine-pad connection point, will block the twine from slipping under the staple during use. The free ends of both lengths of twine are then securely connected to a small loop tied at the end of a similar 5 ft. (1.5 m) length of twine. The resulting assembly resembles the letter Y with a 5 ft. long vertical stem and two diagonal branches (one 1 ft. long and the other 2 ft. long), with a folded ~~swab~~ DS pad securely attached at the end of each branch. ~~After assembly, each two pad drag-swab sampling set is place into a sterile bag.~~ The lengths of twine are wrapped around each 2-pad DS sampler set to produce a small bundle. Assembled DS bundles are then autoclaved and subsequently transferred aseptically (sterile forceps, etc.) to sterile resealble bags. Fifteen ml of double-

strength skim milk[1] is aseptically added to each bag and massaged into the pads. The bags are resealed and stored at -20°C.

(c)(2) Procedures and applications for ~~taking drag-swabs~~ DS samplers.

DS samplers shall be thoroughly thawed prior to use. Complete-Pad-twine-fastener assemblies are used for sampling floor litter surfaces. Nest-box surfaces can be sampled simply using 3 x 3 in gauze pads impregnated with double-strength skim milk as described paragraph (c)(1) of this section. In either instance, sampling personnel must wear fresh sterile disposable gloves between houses and flocks. ~~Floor litter; two samples shall be collected as follows: Four 3 x 3 in. gauze pads pre-moistened with double strength skim milk shall be dragged over the floor litter surface for a minimum period of 15 minutes. The gauze pads and to collect the samples shall be placed in 18 ounces whirl-pak bags, two pads per bag with each bag containing 5 ml. of double strength skim milk. This will maintain the moistness of the sample during transport. The~~ Sampler bags shall be marked with the identity of the type of sample, and the house or flock identification.

(c)(3) Floor-litter sampling technique. For flocks of less than 500 breeders, a minimum of one DS set (2 DS pads) shall be pulled (dragged) across the floor litter surface for a minimum period of 15 minutes. For flocks of 500 or more breeders, a minimum of two DS sets (4 DS pads) shall be dragged across the litter surface for a minimum 15 minute period. Upon completion of dragging, each DS pad is lowered by the attached twine into separate, sterile resealable bags. Alternatively, both pads may be returned to the storage/transport bag from

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[1]Procedure for preparing double-strength skim milk can be obtained for USDA-APHIS Recommended sample collection methods for Environmental Samples available from the National Poultry Improvement Plan staff; Room 771, Federal Building, Hyattsville, MD 20782. Preparation instructions are also available in A laboratory Manual for the isolations and Identification of Avian Pathogens. Chapter 1 Salmonellosis. Third Edition, 1989 American Association of Avian Pathologists, Inc. Kendall/Hunt Publishing Co., Dubuque, Iowa 52004-0539.

which they had originally been taken. the pad(s) and twine are separated by firmly grasping the pad through the sides of the bag with one hand and forcefully pulling on the twine with the other hand until the connection is broken. Reseal bags and promptly refrigerate at 2 to 4°C. Do not freeze. Discard twine in an appropriate disposal bag.

(c)(4) Nest boxes: sampling technique. ~~One~~ Nest-box samples shall be collected by using two sterile 3 x 3 in. gauze pads pre-moistened (impregnated) with double-strength skim milk. The two gauze pads used to collect the samples are forcefully wiped over across assorted locations of approximately 10% of the total nesting area. ~~The gauze pads used to collect the sample shall be placed in an 18 ounce whirl-pak bag containing 5 mils. of double strength skim milk. The bag shall be marked with the identity of the type of sample and house identification.~~ Upon completion, each pad shall be placed into separate, sterile resealable bags. Reseal bags and promptly refrigerate at 2 to 4°C Do not freeze.

(c)(5) Culture of litter surface and nest-box samples. Double-strength skim milk-impregnated pads, when refrigerated as described, may be stored or batched for 5 to 7 days prior to culture. Pads shipped singly or paired in a bag shall not be pooled for culture. Each pad shall be separately inoculated into 60 ml. of selective enrichment broth.

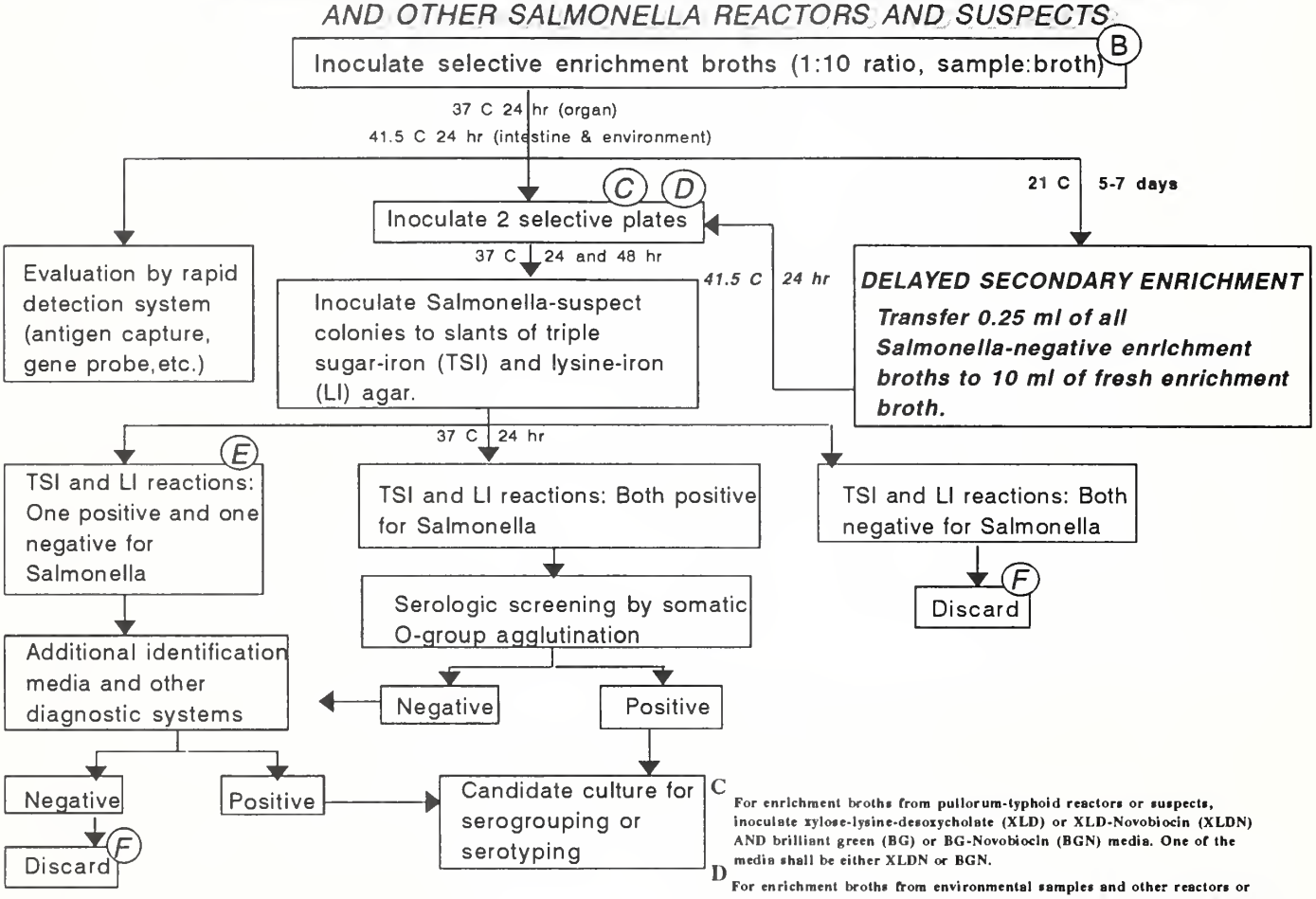
REASON:
The proposed changes seek to provide more clarity and detail for the collection and handling of environmental samples cultured in the NPIP Sanitation Monitored Program. Specific bacteriological techniques for culturing these samples are provided in a flow chart format (Illus. 2) found in proposal 11. Illustration no. 2 updates, and is intended to clarify, earlier NPIP and AAAP specifications.

PROPONENT: Dr. Ed Mallinson, VA-MD Regional College of Veterinary Medicine, University of Maryland, College Park, MD 20742-3711.

Illus. 2

ENVIRONMENTAL, ORGAN AND INTESTINAL SAMPLES (A)

ENVIRONMENTAL MONITORING PROGRAMS AND PULLORUM-TYPHOID AND OTHER SALMONELLA REACTORS AND SUSPECTS



A All reactor and suspect birds must also be evaluated without selective enrichment (Refer to Illus.1)

B Hajna TT or Mueller-Kauffman tetrathionate enrichment broth.

C For enrichment broths from pullorum-typhoid reactors or suspects, inoculate xylose-lysine-desoxycholate (XLD) or XLD-Novobiocin (XLDN) AND brilliant green (BG) or BG-Novobiocin (BGN) media. One of the media shall be either XLDN or BGN.

D For enrichment broths from environmental samples and other reactors or suspects, inoculate xylose-lysine-tergitol 4 or XLDN AND BGN or BG media.

E If combined results with TSI and LI agars, additional identification media, and O-group screening procedures are inconclusive, restreak original colony onto selective plating agar to check for purity.

F Reevaluate if epidemiologic, necropsy, or other information strongly suggest the presence of an unusual strain of Salmonella.

Proposal No. 13

Delegates:

B,C,D,E (combined)

§147.14 PROCEDURES TO DETERMINE STATUS AND EFFECTIVENESS OF SANITATION MONITORED PROGRAM[4]

The following monitoring procedures may be applied at the discretion of the Official State Agency:

(a) no change
(a)(1) no change
(a)(2) Culture a sample of dead-in-shell eggs periodically from each breeding flock for coliforms. ~~The culture media will be designed to include detection of salmonella species.~~ Such eggs should also be cultured for the dependable recovery of salmonellae, including the use of : preenrichment broths, supplemented with 35 gm ferrous sulfate per 5 1000 ml preenrichment to block iron-binding, salmonella-inhibiting effects of egg conalbumin; and tetrathionate selective enrichment broths, competitor-controlling plating media(XLT4,BGN,etc.), and delayed secondary enrichment procedures detailed in Illus. 2.

REASON:
The proposed change adds the recommendation that eggs cultured in the Sanitation Monitored Program first should be cultured in a preenrichment broth supplemented with ferrous sulfate prior to subculture into a tetrathionate selective enrichment. This is consistent with recommendations presented in AAVLD and AAAP laboratory manuals. Ferrous sulfate should be added to egg preenrichments to counteract the salmonella-inhibiting characteristics of egg conalbumin and ovotransferrin. The proposal also provides further direction on culture technique as

[4]American Association of Avian Pathologists, University of Pennsylvania, New Bolton Center, Kennett Square, Pennsylvania 19348-1692.

presented in Illus. 2 and found in proposal No. 11.

PROPONENT: Dr. Ed Mallinson, VA-MD Regional College of Veterinary Medicine, University of Maryland, College Park, MD 20742-3711.

Proposal No. 14

Delegates:
B,C,D,E
(combined)

§145.14 (a) For Pullorum-Typhoid. Amends the provisions as follows:

(a)(6) When reactors are found in any flock, or S. pullorum or S. gallinarum organisms are isolated by an authorized laboratory from baby poultry or from fluff samples produced by hatching eggs, the infected flock shall qualify for participation in the Plan with two consecutive negative results to an official blood test named in paragraph (a)(1) of this section. A succeeding flock must be qualified for participation in the Plan's pullorum-typhoid program with a negative result to an official blood test named in paragraph (a)(1) of this section. Testing to qualify flocks for Plan participation must include the testing of all birds in infected and succeeding flocks for a 12-month period, and shall be performed or physically supervised by a State inspector. At the discretion of the State agency when large flocks are involved, in lieu of the entire flock being tested, a representative number may be tested by the State inspector if it is agreed upon by all involved parties and the administrator. If the State inspector determines that a primary breeding flock has been exposed to S. pullorum or S. gallinarum the Official State agency may require:

(a)(6)(i) The taking of blood samples-performed by or in the presence of a State inspector- from all birds on premises exposed to birds, equipment, supplies, or personnel from the primary breeding flock during the period when the State inspector determined that exposure to S. pullorum or S. gallinarum occurred.

(a)(6)(ii) The banding of all birds of these premises-performed or physically supervised by a State inspector-in order to identify any bird that tests positive; and

(a)(6)(iii) The testing of the blood samples at an authorized laboratory using an official blood test named in paragraph (a)(1) of this section.

REASON:
When large flocks are involved in a pullorum outbreak such as the one we recently experienced, 100% testing of succeeding flocks is very time consuming and costly. We believe in some instances 100% testing is not required to insure pullorum free flocks. For instance, this outbreak involved a parent flock from another state and there was no spread of pullorum to unrelated flocks. A thorough cleaning and disinfecting program on the premises prevented any succeeding flock problems. Pullorum was never isolated from a succeeding flock on any of the former positive premises. If there ever is a suspected problem then a 100% test could be performed. This would save the State agency and the company involved much time and money, since 100% testing places unneeded stress on the chickens.

Proponent:
Mr. Ray Hilburn, Alabama Department of Agriculture, Montgomery, Alabama

Proposal No. 15

Delegates:

C §145.33(b)(5) amends as follows.

(b)(5) It is a primary breeding flock located in a State determined to be in compliance with the provisions of paragraph (b)(4) of this section, and in which a sample of 300 birds from flocks of more than 300, and each bird in flocks of 300 or less, has been officially tested for pullorum-typhoid with no reactors: Provided, That a bacteriological examination monitoring program acceptable to the Official State Agency and approved by the Service may be used in lieu of blood testing, And provided further, That 100 percent of the birds in grandparent male-line primary breeding flocks must be tested.

REASON:
A recent pullorum outbreak indicated that a low-level infection in male-line grandparent flocks may not be detected until the commercial broilers resulting from the progeny male and female parent flocks are produced. This change will help avoid another costly outbreak from occurring.

PROPONENT:
Poultry Improvement Staff

Proposal No. 16

Delegates:

B,C,D,E (combined)

§147.7(f) Procedure for mycoplasma hemagglutination inhibition test using microtiter technique.

Purpose: To test for antibodies to avian mycoplasma by hemagglutination inhibition. The test uses the constant antigen, titered-sera method for measuring antibodies to MG, MS, or MM.

(f)(1) Materials needed.
(f)(1)(i) M. gallisepticum, M. synoviae, and/or M. Meleagridis HI antigens.
(f)(1)(ii) Positive and negative control sera.
(f)(1)(iii) Phosphate buffered saline (PBS).
(f)(1)(iv) Microtiter plates, 96-well, U bottom.
(f)(1)(v) 12-channel pipettor (Titerek).
(f)(1)(vi) 50 µl pipettor (Pipetman P200).
(f)(1)(vii) Pipette tips.
(f)(1)(viii) 0.5% homologous red blood cells in PBS (use RBC's from the same species being tested).
(f)(1)(ix) Plate sealing tape.
(f)(1)(x) Mirrored plate reader.

(f)(2) Microtiter hemagglutination (HA) antigen titration.
(f)(2)(i) Perform standard hemagglutination test (HA) on mycoplasma antigen to determine titer of antigen.
a) Dispense 50 µl of PBS into each well of 3 rows of a 96-well microtiter plate.
b) Dispense 50 µl of stock antigen into the well of 2 rows.
c) Perform serial two-fold dilutions (50 µl) using a 12-channel pipettor. The dilution series will be from 1:2-1:4096.
d) Add 50 µl of 0.5% homologous RBC's to each well of all three rows. The row with no antigen serves as a RBC control.
(f)(2)(ii) Incubate at room temperature (approximately 30 minutes) until the control RBC's give tight

buttons. The HA titer is read as the last well to give a complete lawn (hemagglutination). The desired endpoint is 4 HA units. The well containing the 1:4 dilution should give a complete hemagglutination while the 1:8 dilution should show less than complete HA.

(f)(2)(iii) Dilute stock antigen to 4 HA units for the HI test. The dilution required to give 4 HA units is calculated by dividing the stock antigen HA titer by 8:

Example: 1:320 HA units/8 = 40, dilute stock antigen 1:40.

(f)(3) Hemagglutination inhibition assay:

(f)(3)(i) Label one column (A-H) of a 96 well, U bottom microtiter plate for each sample, each positive and negative control sera, antigen backtitration and RBC control.

(f)(3)(ii) Add 40 µl of PBS to the top row of wells (row A) of the plate.

(f)(3)(iii) Add 25 µl of PBS to all remaining wells of the plate.

(f)(3)(iv) Add 10 µl of each test sera to well A of each column (making a 1:5 sera dilution).

(f)(3)(v) Serially dilute 25 µl from well A through H using a 12-channel pipettor. Discard the final 25 µl. Row A=1:5...row H=1:640.

(f)(3)(vi) With an Oxford doser, add 25 µl of 4 HA unit antigen to wells B through H. Well A serves as sera control.

(f)(3)(vii) Prepare an antigen backtitration by adding 25 µl of PBS to each well of one column. Add 25 µl of diluted antigen to well A and serially dilute 25 µl from well A-D. This prepares 1:2, 1:4, 1:8, and 1:16 dilutions. (It is recommended that the antigen control backtitration be performed before the diluted antigen is used in the assay. Dilution problems could be detected

and corrected before the inappropriately diluted antigen is used in the assay).

(f)(3)(viii) Leave a column of wells blank for a RBC control.

(f)(3)(ix) Agitate gently and incubate for 30 minutes at room temperature.

(f)(3)(x) Add 50 μl of 0.5% RBC's to all wells. Note: Do not agitate after RBC's have been added (agitation may result in false positive reactions by causing the RBC's to fall, resulting in "false" buttons).

(f)(3)(xi) Cover the plate with sealing tape. Incubate at room temperature (approximately 30 minutes) until control RBC"s give a tight button.

(f)(3)(xii) Read the reaction on a mirrored plate reader.

(f)(4) Results: The titer is reported as the reciprocal of the last dilution to give a TIGHT BUTTON OF RBC'S. The final dilution scheme includes the antigen in the dilution calculation and is as follows: B=1:20, C=1:40, D=1:80, E=160, F=1:320, G=1:640, H=1:1280.

For the assay to be valid:
The positive control sera must give a result within one dilution of the previously determined titer.
The negative control sera must be negative.
The backtitration of the antigen must be 1:4 or 1:8.
The RBC control must give tight, non-hemolyzed buttons.
Sera controls (well A of each test sera) must not have non-specific agglutination or hemolysis. If negative, report as "negative with non-specific agglutination or nonspecific hemolysis" or "unable to evaluate due to non-specific agglutination or hemolysis."

Or treat the serum to remove the non-specific agglutination and

repeat the test. See "Serum Treatment to Remove Non-Specific Agglutination" protocol.

(f)(5) Treatment To Remove Non-Specific Agglutination.

Purpose: Treatment of serum to remove non-specific agglutination that is interfering with hemagglutination-inhibition (HI) assays.

(f)(5)(i) Specimen: Serum

(f)(5)(ii) Materials: Homologous red blood cells (chicken or turkey), 50% solution phosphate buffered saline (PBS), centrifuge, incubator, 4C (refrigerator).

(f)(5)(iii) Procedure:

1) Prepare a 1:5 dilution of test serum by adding 50 µl of serum to 200 µ of PBS.
2) Prepare a 50% solution of RBC's by adding equalvolumes of packed RBC's to PBS. Mix well.
3) Add 25 µl of 50% RBC solution to the serum dilutions.
4) Vortex gently to mix.
5) Incubate at 4 C for 1 hour.
6) Centrifuge to pellet the RBC's.
7) Use the supernatant to perform the HI assay. Modify the dilution scheme in th assay to consider the initial 1:5 dilution prepared in the treatment. For the 1:5 dilution scheme, do not add PBS to row A. Add 50 µl of the 1:5 treated supernatant to row A. Serially dilute 25 µl from row A through H. This prepares a serum dilution of 1:10 through 1:640 in rows B through H.

Reason:
The assay differs from the protocol outlined in the National Poultry Improvement Plan and Auxiliary Provisions in the following ways.

1). A single 4 HA dilution of antigen is prepared and used for all serum dilutions. The current procedure requires preparation of 8 HA units and a 4 HA units of antigen.
2). Serial two-fold dilutions of serum samples are made prior to addition of 4

HA units of antigen to all dilutions. The current procedure adds 8 HA units of antigen to the first dilution (highest antibody concentration), and serially dilutes the antigen-antibody mixture into tubes or wells containing 4 HA units of antigen.

PROPONENT:
Dr. Sharon K. Hietala
CVDLS Immunology
University of California, Davis
Davis, California

Proposal No. 17

Delegates:

B

§ 145.23 (h) **U. S. S. ENTERITIDIS MONITORED STARTED POULTRY**

(h) **U. S. S. ENTERITIDIS MONITORED STARTED POULTRY**-This program is intended to be the basis from which the started-poultry industry may conduct a program for the prevention and control of Salmonella enteritidis. It is intended to provide reasonable assurance to egg producers that a pullet flock may be certified for a quality assurance S. enteritidis laying flock program.

(h)(1) A pullet flock which has met the following requirements as determined by the Official State Agency:

(h)(1)(i) Replacement chicks shall originate from parent flocks and hatchery which meet the requirements of U.S. Sanitation Monitored for egg type breeders or an equivalent program.

(h)(1)(ii) Baby chicks shall be placed in cleaned and disinfected houses that have been environmentally examined and are negative for Salmonella.

(h)(1)(iii) The flock is maintained in compliance with §§ 147.21, 147.24 (a), and 147.26 of this chapter;

(h)(1)(iv) Environmental samples shall be collected from the grow-out facility by an Authorized Agent, as described in § 147.12 of this chapter, at 7-10 days of age and at 12-18 weeks of age. The samples shall be examined bacteriologically for group D salmonella at an authorized laboratory. Cultures from positive samples shall be serotyped.

(h)(1)(v) Approved rodent, wild bird and fly control programs will be required in the pullet growing facility.

(h)(1)(vi) All feed fed to the flock shall meet one of the following requirements:

(h)(1)(vi)(A) Pelletized feed shall contain either no animal protein or only animal protein products produced under the Animal Protein Products Industry (APPI) Salmonella Education/ Reduction Program, a minimum moisture content of 14.5 percent, and must have been subjected to temperature of 190°F. or above, 165°F. for at least 20 minutes, or

184°F. and 70 lbs. of pressure during the manufacturing process.
(h)(1)(vi)(B) Mash feed shall contain either no animal protein or only animal protein product supplements manufactured in pellet form and crumbled.
(h)(1)(vii) Feed shall be stored and transported in such a manner as to prevent possible contamination;
(h)(1)(viii) A sample of early mortality shall be collected at 7-10 days. The samples shall be examined bacteriologically for group D salmonella at an authorized laboratory. Cultures from positive samples shall be serotyped
(h)(1)(ix) Pullets shall be transported only in cleaned and disinfected crates and vehicles.
(h)(2) Isolation of SE from an environmental or other specimen (early chick mortality) as described in section (h)(1)(iv) or (h)(1)(viii) of this paragraph will require the bacteriological examination of 60 birds from the flock for Salmonella enteritidis in an authorized laboratory as described in section 147.11 of this chapter.
A flock shall not be eligible for this classification if Salmonella enteritidis (S. enteritidis ser Enteritidis) is isolated from the internal organs (heart, liver, gall bladder, spleen, oviduct, and ovary) of a bird in the flock.
(h)(3) A flock shall remain eligible for this classification if Salmonella enteritidis (S. enteritidis ser Enteritidis) is only isolated from the environment.
(h)(4) This classification may be revoked by the Official State Agency if the participant fails to follow recommended corrective measures.

REASON:
This is a voluntary program for egg producers or others who wish to have reasonable assurance at to the SE status of pullets which are being added to their laying flocks. This program, although not a program involving breeding flocks or hatching is very similar to current pullet programs of NPIP which classify pullets in regard to MG and MS.

This program providing documented and approved guidelines for the industry to follow in establishing SE control programs of their own even though not necessarily supervised and classified by an Official State Agency.

Having such a voluntary program available will tend to deemphasize any perceived need for mandatory program.

PROPONENT:
Irvin L. Peterson, DVM, Senior Staff, Veterinarian, VS, APHIS, USDA.

Proposal No. 18

Delegates: B,C,D,E (combined)

§ 147.11 LABORATORY PROCEDURE RECOMMENDED FOR THE BACTERIOLOGICAL EXAMINATION OF SALMONELLA REACTORS. Amends as follows:

(a) Grossly normal or diseased liver, heart, pericardial sac, spleen, lung, kidney, pancreas, peritoneum, drained gallbladder or oviduct; misshapen ova or testes; inflamed or unabsorbed yolk sac; and other visibly pathological tissues where purulent, necrotic, or proliferative lesions are seen (including cysts, abscesses, hypopyon, and inflamed serosal surfaces), should be directly cultured by means of a flamed wire loop or with sterile swabs.[1] Careful aseptic technique must be utilized throughout the process of collecting tissues. Selective media should not be relied upon to deal with contaminants, since some strains may not dependably survive and grow in certain selective media. Inoculate veal infusion (VI) and brilliant green (BG) agar plates. Incubate the plates for 24 and 48 hours at 37°C. The digestive system should always be cultured separately (see paragraph (f) of this section) after other anatomical organs and systems have been collected and cultured.

(b) The following organs should be aseptically collected for culture:

(b)(1) Heart (apex, pericardial sac, and contents if present.

(b)(2) Liver (portions exhibiting lesions or, in grossly normal organs, the drained gallbladder and adjacent liver tissues.);

(b)(3) Ovary-Testes (entire inactive ovary or testes, but if ovary is active, use own judgment and include any atypical ova.);

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[1]Culture media preparation and biochemical identification charts can be obtained from Culture Methods for the Detection of Animal Salmonellosis and Arizonosis, Committee on Salmonellosis and Arizonosis; AAVLD; 1976 Iowa State University Press; Ames, Iowa 50010.

(b)(4) Oviduct (if active, include any debris and dehydrated

(b)(5) Pancreas and kidneys; and

(b)(6) Spleen.

(c) Aseptically collect 10-15 g or whatever lesser amount is available of each organ or site listed in paragraph (b) from each reactor, and grind or blend them completely in 10 times their volume of VI broth. Organs may be processed individually or in combinations where appropriate. Suspensions should be transferred in 10 ml aliquots to 100 ml of both VI and ~~tetrathionate brilliant green (TBG)~~ Tetrathionate Hajna (TTH) broth and incubated at 37°C. for 24 hours. Plate the VI broth on VI and BG agar and plate the ~~TBG~~ TTH broth on BG agar and incubate at 37°C. Examine these plates after 24 and 48 hours of incubation. The contents of the gallbladder can be cultured separately by inoculating 10 ml of VI and ~~TBG~~ TTH broth with cotton swabs and incubating at 37°C. for 24 hours. Plate on BG agar and incubate at 37°C. Examine these plates after 24 and 48 hours of incubation. If contamination with pseudomonas or proteus is a problem, make platings on ~~BG sulfapyridine (BGS)~~ BG Novobiocin (BGN) agar (see Illus 3).

(d) Where field samples are directly inoculated into enrichment broths and a delay of several days occurs before they reach a laboratory, or if recovery of low numbers or organisms is expected from a primary culture, a secondary enrichment culture should be prepared. Subculture a week-old primary culture by transferring 1 ml of inoculum into a fresh tube containing 10 ml of enrichment broth. This secondary enrichment should be incubated at 37°C. for 24 hours before plating. (See paragraph (a) of this section.) ~~TBG~~ TTH broth is recommended for this procedure.

(e) Make a composite sample of the following parts of grossly normal or diseased tissues from the digestive tract: Crop wall, duodenum, jejunum (including remnant of yolk-sac attachment), both ceca, cecal tonsils, and rectum-cloaca. Aseptically collect 10-15 g or

whatever lesser amount is available of each organ or tissue and grind or blend them completely in 10 times their volume of TBG broth. Transfer 10 ml of a composite sample of a suspension from the digestive tract into 100 ml of ~~TBG~~ TTH broth, and incubate flasks at 42°C. for 24 hours. Cultures may be incubated at 37°C. if 42°C. incubators are not available. The higher incubation temperatures for ~~TBG~~ TTH broth reduce populations of competitive contaminants common in gut tissue. Plate on BG agar and incubate at 37°C. Examine the plates after 24 and 48 hours of incubation. If contamination with pseudomonas or proteus is a problem make plating on ~~BGS~~ BGN agar.

(f) If preferred, individual cotton swab samples may also be taken from the upper, middle, and lower intestinal tract (including both ceca and the rectum-cloaca). Deposit swabs in 10 ml of ~~TBG~~ TTH broth and incubate and plate as described in paragraph (e) of this section.

(g) Transfer suspect colonies to triple-sugar-iron (TSI) agar and lysine-iron (LI) agar and incubate at 37°C. for 24 hours.

(h) Cultures revealing typical reactions of salmonellae on TSI and LI agar slants should be transferred to any of the following appropriate biochemical tests for final identification: Dextrose, lactose, sucrose, mannitol, maltose, dulcitol, malonate, gelatin, urea broth, citrate, lysine decarboxylase, ornithine decarboxylase, methyl red and Voges-Proskauer, KCN, salicin broths, indole, and hydrogen sulfide. Motility or non-motility is demonstrated by inoculating a suitable semisolid medium.[2] The Analytical Profile Index for Enterobacteriaceae (API) system may be utilized for identification if feasible. For arizonae identification, make readings daily up to 10 days. An O-nitrophenyl-beta-D-galactopyranside (ONPG)

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[2]A Laboratory Manual for the Isolation and Identification of Avian Pathogens (Third Edition) 1989. Amercian Association of Avian Pathologists; University of Pennsylvania; New Bolton Center; Kennet Square, Pennsylvania 19348-1692.

disc may be used to identify slow lactose fermenters.[3]

(i) All salmonella cultures should be serologically typed.

REASON:

Updating the current procedures in the NPIP and Auxiliary Provisions VS 91-40, August 1989 by substituting the use of TT Hajna broth for TBG broth and BGN plates for BGS plates.

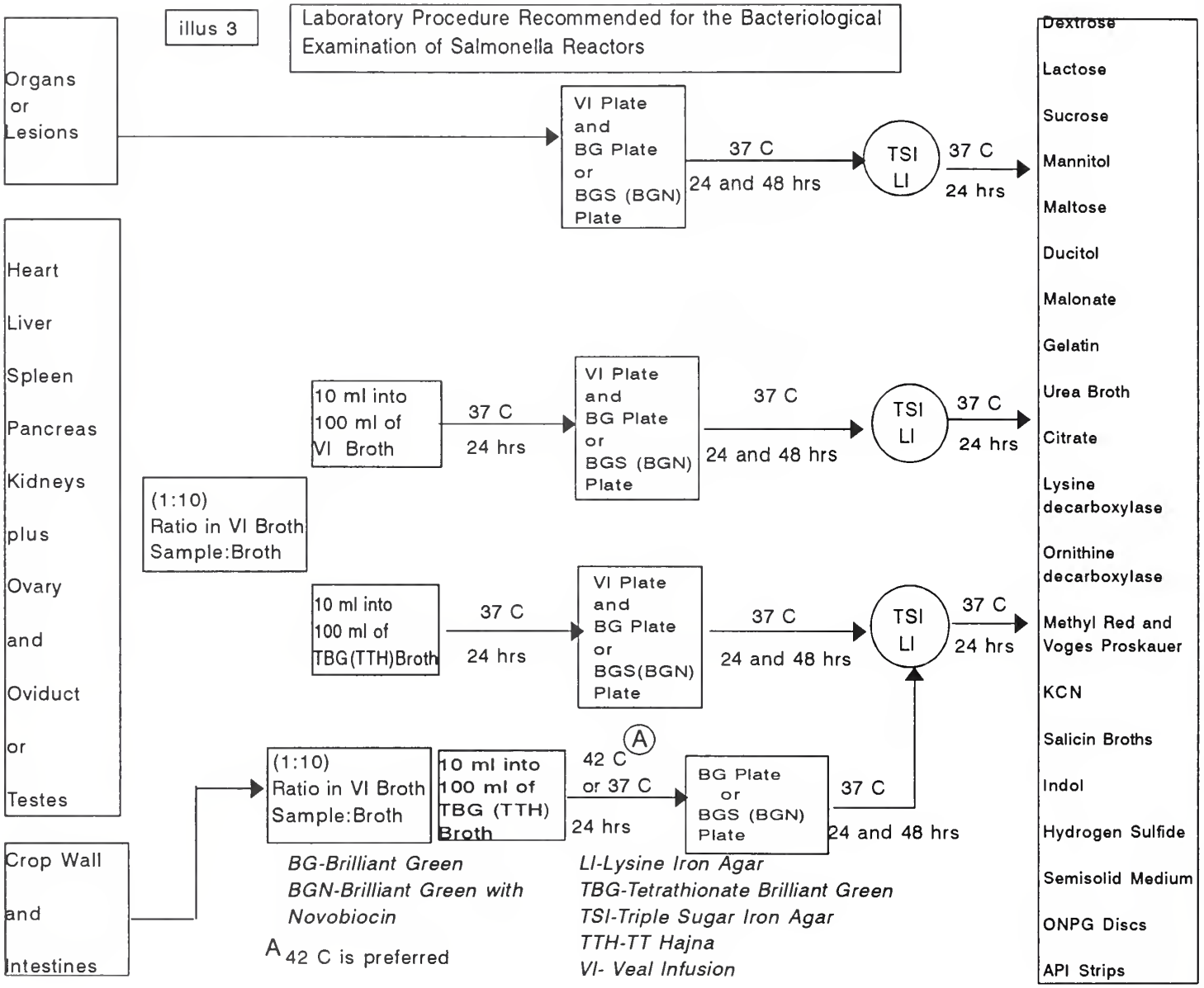
PROPONENT:

Dr. George Stein, Jr.
Maryland Department of Agriculture
Animal Health Laboratory
P.O. Box J
Salisbury, MD 21802

[3]ONPG discs are available from: Baltimore Biological Laboratories; Cockeysville, Maryland 21030.

illus 3

Laboratory Procedure Recommended for the Bacteriological Examination of Salmonella Reactors



BG-Brilliant Green
BGN-Brilliant Green with Novobiocin

A 42 C is preferred

LI-Lysine Iron Agar
TBG-Tetrathionate Brilliant Green
TSI-Triple Sugar Iron Agar
TTH-TT Hajna
VI- Veal Infusion

Proposal No. 19

Delegates:
B

§ 145.23 (d) U.S. SANITATION MONITORED.
Amends as follows:

§ 145.23 (d) U.S. ~~SANITATION MONITORED~~ S. enteritidis Monitored.

Reason:
The classification "U.S. Sanitation Monitored" is currently used for both meat and egg-type chickens, although the requirements for the two are vastly different. To avoid confusion the two classifications should have different names.

Proponent:
W. Douglas Waltman II, Ph.D. Georgia Poultry Laboratory, Oakwood, Georgia

Proposal No. 20

Delegates:
B,C,D,E

§145.12 INSPECTIONS amends as follows:

(a) Each participating hatchery shall be inspected a sufficient number of times each year to satisfy the Official State Agency so that the operations of the hatchery are in compliance with the provision of the plan.

(b) The records of all flocks maintained primarily for production of hatching eggs shall be examined annually by a State inspector. Records shall include froms VS 9-2 and VS 9-3, set and hatch records, egg receipts and egg/chick orders or invoices. Records shall be maintained in an organized and accessible manner for three (3) years. On-site inspection of flocks and premises will be conducted if the State inspector determines that a breach of sanitation, blood testing or other provisions have occurred for Plan programs for which the flocks have or are being qualified.

Purpose:
The purpose of this proposed change is to clearly define the term "records" as it applies to hatchery records of hatcheries operating under the National Poultry Improvement Plan and to establish a uniform time period for which such records will be maintained. This change will improve the quality of the investigation of a disease break whether the break occurs within the State or in another State. Some States may cover this in State regulations, however, that does not insure that all Plan States will have a uniform minimum standard.

Proponent:
Ms. Rose Foster
State Livestock Inspector
Missouri Department of Agriculture
Jefferson City, MO